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Selection and propagation of hybrid aspen clones for growth and fibre quality

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ACADEMIC DISSERTATION IN FOREST TREE BREEDING

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Dedicated to my departed grandmother, Xie Deyun (1906-1989)

CONTENTS

Abstract

Hybrid aspens between *P. tremula* and *P. tremuloides* are fast growing, and have fibres of narrow diameter and thin walls which are ideal for producing a high-density paper sheet with a smooth surface. The work reported in this thesis was a subproject of a consortium entitled “aspen in paper making”, which is one of the research programmes in Wood Wisdom, Finnish Forest Cluster Research Programme. The main objectives of the present study are to understand better the genetic control of wood physico-chemical properties in hybrid aspen, the genetic relationships of these wood properties with growth and phenological traits, and to find new and practical approaches to utilizing genetic gain.

The materials used in the present study comprise seven hybrid aspen clonal trials in Finland and Sweden, as well as newly propagated hybrid aspen clones in Haapastensyrjä Nursery, Finland.

Significant differences were found among hybrid clones in wood physico-chemical properties, in physiological, morphological and phenological characters, and also in rooting ability. Relatively high repeatabilities for wood quality characters and growth traits were found. Among the physiological and morphological characters, only stomatal characters of hybrid clones differ from those of *P. tremula*. The high stomatal density and small mean guard cell length in *P. tremula* may be associated with the adaptation of this species to long day-lengths during the summer. A strong correlation was found between foliar nitrogen and net photosynthesis among the hybrid clones.

The fast overall growth of the hybrid aspens is largely explained by their longer vegetative period. This response is not true heterosis as has been commonly assumed. Annual growth rhythm is an important determinant of variations in wood physico-chemical properties. The length of the growth period should be a good predictor of clonal differences in fibre count. The estimated correlations were unfavourable, in the sense that clonal selection directed at increasing the fibre count is expected to produce an indirect genetic decline in growth. Inter-site genetic correlations indicated that wood characters were more stable than growth traits. A statistically significant interaction in height growth exists between aspen clones and their test sites. The ranking of clones changed across sites, indicating significant site x clone interactions in height and basal diameter. The topography (slope), soil structure and nutrient availability appear to be contributing to the main environmental factors interacting with genotypes in southern Finland.

Stem cuttings were harvested from the same micropropagated donor plants twice during the growing season. A significant variation for most traits related to rooting ability was found among the clones, indicating that clonal effects play an important role in the propagation of aspen. Thus, clones with a good response in shoot growth and rooting could be exploited in large-scale propagation by stem cuttings.

A breeding strategy for hybrid aspens is proposed.

LIST OF ORIGINAL PUBLICATIONS

I. Yu, Q., Tigerstedt, P.M.A. and Haapanen, M. 2001. Growth and phenology of hybrid aspen clones (*Populus tremula* L. *x Populus tremuloides* Michx.). Silva Fennica, 35 (1): 15-25.

II. Yu, Q. 2001. Can physiological and anatomical characters be used for selecting high yielding hybrid aspen clones? Silva Fennica, 35 (2): 137-146.

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IV. Yu, Q. and Pulkkinen, P. 2001. Genotype-Environment interaction and stability in growth of aspen hybrid clones. Submitted to Forest Ecology and Management.

V. Yu, Q. Mäntylä, N. and Salonen, M. 2001. Rooting of hybrid clones of *Populus tremula* L. x *P. tremuloides* Michx. by stem cuttings derived from micropropagated plants. Scandinavian Journal of Forest Research. 16(3): 238-245.

1. INTRODUCTION

1.1. Biology and distribution

Poplar and aspen belong to the genus *Populus*, family Salicaceae. They are dioecious, with male and female flowers (in catkins) occurring on separate trees. The diploid chromosome number in poplar and aspen is 2n = 38. The genus *Populus* comprises five sections, consisting of more than 35 species widely distributed in the Northern Hemisphere. In the present context we are concerned with the section *Leuce*, a large group further divided into two subsections: *Albidae* consisting of white poplars, *P. alba* L. and *P. tomentosa* Carr., and *Trepidae*, comprising of the aspens.

P. tremula has an immense distribution, even greater than that of *Pinus sylvestris*. It is native to Finland and covers almost the whole of Europe and western Asia, extending in the north and east through Siberia to the Bering Sea and in the south into North Africa. In Finland, it is a widely distributed species, often growing in mixed stands with pine, spruce and birch. It tolerates a wide range of soil types including heavy clay soils and freely drained sands and gravels. Finnish dendrological literature recognises five forms and three cultivars of aspen in Finland. The most common cultivar in Finland is 'Erecta', which is often planted along streets and by roadsides (Piirainen 1996).

The best known hybrid aspen is between *P. tremula* and the North American *P. tremuloides*. The first controlled crosses were made in Sweden in the 1940s and Denmark. Commercial plantings are presently confined to Scandinavia where there is less bacterial canker than in the rest of Europe (Jobling 1990).

1.2. Economic importance and breeding history in Finland

Considerable genetic gains from aspen hybrids have been demonstrated in Finland (Beuker 1989). The annual height increment of hybrid aspen can be up to one meter per year and 300 m^3/ha during the first 25 years, which is approximately more than 30% for silver birch, 100% for conifers and 50% for native aspen (Hynynen and Kaksson, 2001). The normal 40-50 year rotation for unimproved aspen could be reduced to 20-30 years by the use of hybrids.

Hybrid aspens are fast growing, and their white coloured wood constitutes a premium in paper manufacture where there is pressure to reduce the use of chemicals. The process saves on production costs and is less harmful to the environment. Since aspen is low in lignin and high in carbohydrates, its wood is amenable to many kinds of chemical or mechanical pulping (Karl 1988; Macleod 1987). Aspen has thin-walled fibres of narrow diameter which are ideal for producing a high-density paper sheet with a smooth surface (Karl 1988; Dhak et al. 1997). Aspen fibres will give magazine paper of better rigidity and opacity than other tree species in Finland. In addition it reduces paper weight (Ranua 1996). In countries with highly developed paper-making techniques, the demand may be for uniformly short fibres. In the future, the paper-making industry may require rapidly growing trees harvested in short rotations.

Finally, I think hybrid aspens may be well suited to the reforestation of fertile surplus farmland.

In 1950, the first crosses between *P. tremula* and *P. tremuloides* were made at the Ruotsinkylä field station of the Finnish Forest Research Institute. During the fifties and the sixties, hundreds of crosses were made. The progenies of the crosses were planted out in 17 trials and in 670 stands all over the country. Later, a few F2 hybrids were also produced and planted in trials (Beuker 1989). In the beginning of the 1970s, 300 of these stands, comprising more than 170 families and more than 60 000 trees, were measured and evaluated. At that time, the Finnish match industry was interested in aspen and poplar wood. Subsequently, due to the decline of the match industry during the early 1970s, interest in aspen and poplars in Finland waned. Recently, the forest industry has shown a fresh interest in utilizing aspen for pulp production, and a significant increase in aspen use is expected. Since 1995, from these plantations of aspen hybrids, over 400 aspen and hybrid aspen have been selected on the basis of the phenotype, e.g. growth and quality (Pulkkinen 2001), but less than 50 clones were selected for vegetative propagation after analyses of physico-chemical properties.

1.3. Hybridization for improvement of productivity

Hybrid vigour has been observed in poplar and has been one of the driving forces in poplar breeding (Muhle Larsen 1970). The phenomenon is also well documented for aspen hybridization in the US. Heterosis is claimed to be due to over-dominant interaction in F1 between *P. tremuloides* and *P. tremula* (Li and Wu 1996, 1997; Li et al. 1998).

1.3.1. Rooting ability

Rooting ability of stem cuttings is an important trait in the multiplication of hybrid clones.The rooting of *Populus deltoides* has been significantly improved by crossing it with the well-rooting *Tacamahaca* poplars. Rooting genes introduced from *P. alba* have been used in advanced generation hybrids with *P. grandidentata*, *P. tremuloides*, P. *tremula and P. davidiana* (Heimburger 1968, Zsuffa 1979).

1.3.2. *Growth*

This increase in vigour is likely to depend on many components, including carbon allocation patterns, water and nutrient use efficiency, and shoot growth phenology (Hinckley et al. 1989). Traits that affect *Populus* performance include phenology (Michael et al. 1990, Ceulemans et al. 1992), leaf and stomatal morphology and photosynthetic capacity (Hinckley et al. 1989). Large clonal differences in phenology, leaf area development and photosynthetic production have been observed (Ceulemans et al. 1987; Orlovic et al. 1998; Thomas et al. 1997a and 1997b). Li et al. (1998) found that interspecific hybrid aspens grew faster than intraspecific hybrids at the juvenile stage. The authors ascribed this to larger internode numbers and length and leaf numbers. They also found that the vigour growth of *P. tremula x P. tremuloides* hybrids for stem volume is probably the result of delayed bud set, which results in a longer duration of height growth.

Leaves of *P. deltoides x P. trichocarpa* hybrids are approximately twice the size of leaves of either species. The increase results from larger cell numbers combined with large cell size (Ridge et al. 1986). This combination in F1 hybrids correlated with higher productivity in short rotation plantations (Ceulemans et al. 1992).

1.3.3. Wood quality

Wood density (or specific gravity) is considered to be one of the most important quality properties, as high density has a positive effect on most of the final products of wood (Zobel and Talbert 1984). However, for aspen it has been claimed that fibre properties may adequately predict the quality of resulting fine paper (Ranua 1996).

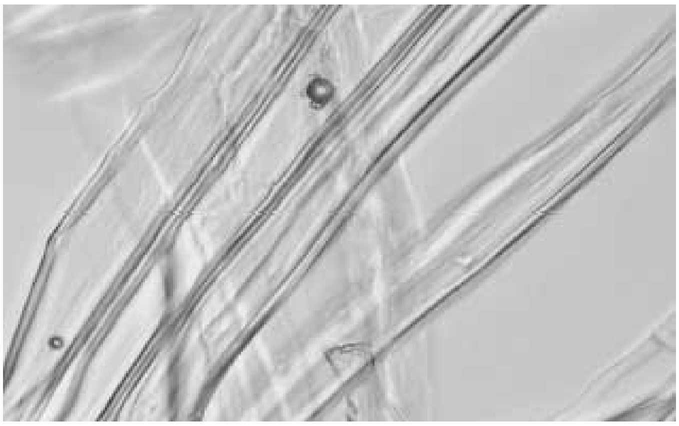
Dinus et al. (2001) found genetic variation in lignin content among and within poplar species, and its heritability exceeded that of growth rate. Tarvainen (1999) reported that the fibre properties affecting paper quality have been found to vary considerably among more than 800 hybrids and pure aspens. The fibre length of hybrid aspens varied within the range of 0.62-1.12 mm, with the average value being 0.86 mm. The corresponding values for domestic aspen were 0.82-1.19 mm and 0.99 mm. Figure 1 shows an example comparing a pure aspen clone (photo a) with a hybrid clone (photo b) (Rautio 1999).

There is limited information comparing hybrid aspen and pure aspen for physico-chemical quality characters. Aziz et al. (1996) studied wood and pulp properties of 27-year-old aspen and 19 year old hybrids. They found that the triploid hybrid had the longest fibres and greatest wood density. The coarseness of hybrid aspen is similar to that of quaking aspen, but triploid aspen has the highest coarseness. Hybrid aspen pulp sources were slightly higher in extractives than the native aspen.

Lignin is mainly found in walls of xylem cells such as tracheary elements and xylary fibres, which contribute up to 15% to 35 % of the dry weight of wood. Lignin ranks the second most abundant components of biomass on earth after cellulose. Wood raw materials which contain low concentrations of easily extractable lignin and less lignin will be needed in the future, because these materials require less drastic cooking and bleaching processes for paper production. Ferrari (1987) reported a heritability value for lignin of 0.75 among nineteen 12-year-old *P. X euramericana* [*P. canadensis*] clones. So it may well be rather easy to improve this character by selection.

According to my knowledge, there is no previous information available on the variation and inheritance of lignin for aspen or their hybrids. However, there are sufficient studies on the possibilities to reduce the concentration of aspen lignin by molecular techniques (e.g. Hu et al 1999; Zhong et al 2000). The basic features of many enzymes in the lignin biosynthetic pathway are known (Campbell and Sederoff 1996). Hu et al. (1999) reported the generation of 25 transgenic aspen (*Populus tremuloides*) lines in which a recently isolated lignin biosynthetic pathway gene Pt4CL1 was expressed. Trees with suppressed Pt4CL1 expression exhibited a reduction in lignin of up to 45%, but this was compensated for by a 15% increase in cellulose. As a result, the total lignin-cellulose mass remained essentially unchanged.

a



b

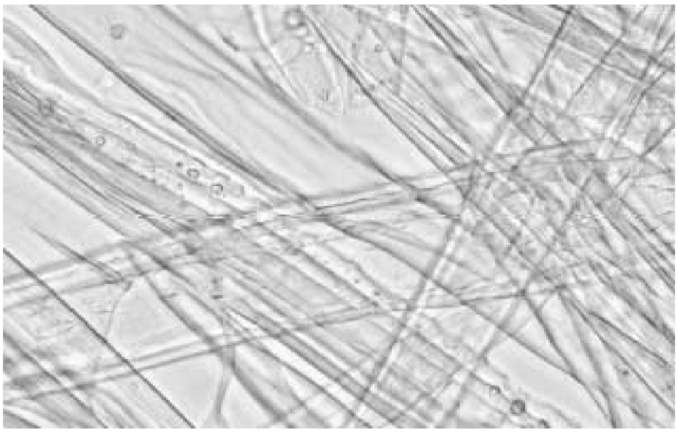


Figure 1. Two different aspen hybrid clones under the microscope. A pure aspen clone (a) gave a low fiber count (3.46 x 10^6 fibers/g pulp), but had long and strong fibers, whereas a hybrid clone (b) give a higher fibre count (14.99 x 10^6 fibers/g pulp), but short and fragile fibers (Mari Rautio, 1999).

1.4. The effect of growth on wood quality

Any factor affecting the physiology and growth of a tree can influence wood quality (Zobel and Talbert, 1984). In addition to wood density and fibre dimensions, such characteristics include cellulose, hemicellulose, lignin and soluble constituents, as well as elemental composition and the presence of aromatic lignin-based esters (Alén 2000). Higher wood density was correlated with phenological traits e.g. cambial growth rhythm, timing of diameter growth, in young *Pseudotsuga menziesii* var. *menziesii* (Vargas-Hernandez and Adams 1994) or shoot flushing and elongation in *Picea abies* (Skrøppa et al. 1999). The correlations of wood density with growth traits were negative in black spruce (Zhang and Morgenstern 1995) and white spruce (Corriveau et al. 1991). Weak positive correlations were found between wood density and growth rate in *Populus x euramericana* (Beaudoin et al. 1992). However, a slight negative correlation was reported between wood density and growth rate in clones of *P. tremuloides* (Yanchuk et al. 1984). In a study on hybrid aspen between *P. tremula* and *P. tremuloides*, there was no tendency for trees of high volume growth to have low wood density (Ilstedt and Gullberg 1993).

Some studies report that fibre length increased with faster growth (Johnson 1942; Kennedy 1957; Kennedy and Smith 1959; Yanchuk et al. 1984). Most of these studies were conducted with wood samples collected in natural stands, and growth rate differences were generally associated with differences in genotype (different stands), geographic location or sites. One report on a hybrid poplar (*P. trichocarpa* x *P. deltoids*) test plantation indicated that fibre length within rings produced at a given age is unrelated to growth rate of during the first six years of growth, but that subsequent fast growth will most likely affect mean fibre length within rings, as a greater proportion of wood is contained in older (outer) rings of fast-growing trees than in slow-growing trees (DeBell et al 1998).

1.5. Vegetative propagation

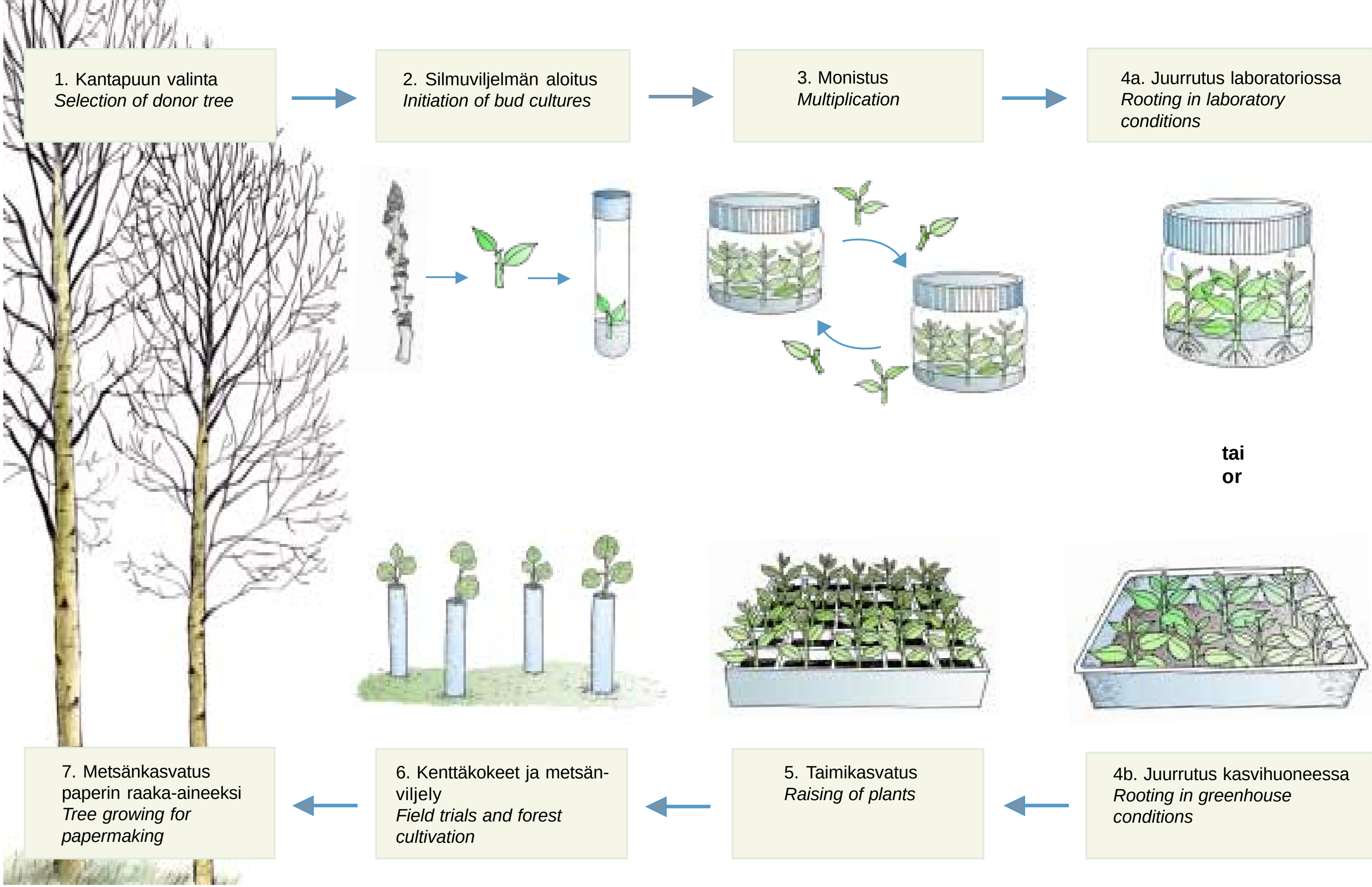
Aspen often regenerates vegetatively under natural conditions. It has the ability to form root suckers, and one often finds small monoclonal stands consisting of hundreds of ramets from a single ortet. This natural “cloning” ability has led breeders to use vegetative propagation as an adjunct to breeding work. Traditional tree improvement programmes based on recurrent selection and seedling propagation can utilize only additive genetic variation, whereas a clonal selection approach capitalizes on the total genetic variation. Aspen hybrid clones with uniform short wood fibres can thus be deployed for producing a valuable industrial raw material.

Poplar species are often propagated vegetatively from stem cuttings. However, aspen stem cuttings root very poorly (Maini 1968). Vegetative propagation of selected aspen clones has also been attempted by means of root suckers and green shoots (Farmer 1963, Zufa 1972). Results have been variable, but the aspen’s generally low rooting ability results in a high plant cost which precludes practical utilization.

Over the last two decades, other more sophisticated techniques, such as micropropagation and somatic embryogenesis, have been investigated for large-scale propagation of aspen (Ahuja 1983, 1984; Michler and Bauer 1991). However, micropropagated planting stock is even more expensive than cuttings or seedlings. The high costs of micropropagation are associated with the high labor input, expensive facilities and a high energy requirement (Vasil 1994, Lian and Khayat 1997). In order to reduce the high labor cost, and to avoid using expensive facilities, ways of transferring vegetative propagation procedures from the laboratory to the greenhouse need to be examined more carefully.

The current commercial scale of vegetative propagation procedures for hybrid aspen in Haapastensyrjä nursery is presented in Figure 2, which was published in the annual report 1998 of Foundation for Forest Tree Breeding.

HAAVAN TAIMIEN TUOTTAMINEN MIKROLISÄYKSEN AVULLA
PRODUCTION OF MICROPROPAGATED ASPEN



2. Objectives

The main objectives of the present study are to understand the physio-genetic basis of wood quality and high yield in hybrid aspen clones and to find new and practical approaches to utilizing the genetic gain. To meet these objectives, studies were carried out to characterize the genetic variations of wood quality related characters in aspen hybrids, and to correlate these components with growth and phenological traits.

The following hypotheses are considered during the study:

1. Large differences in wood quality and in physiological and morphological characters can been found among clones representing various hybrid combinations.

2. Wood quality characters are sometimes negatively correlated with growth related characters, but outliers allow selection of high-yielding clones for industrial purposes.

3. The high yield of hybrid clones is due to their extended growth period.

4. There is a GxE interaction for both growth and wood quality traits.

5. Rooting of hybrid clones by stem cuttings provides a means of reducing costs during vegetative propagation of aspen clones.

3. Materials and Methods

3.1. Plant materials

The materials used in the present study comprised: (1) hybrid clones between *P. tremula* and *P. tremuloides* from seven field trials in Sweden and Finland (Table 1), (2) hybrid clones derived from micropropagated plants for stem cutting experiments (Paper V).

Table 1. Field trials used in the study

Trial No.	Year Established	No. of Clone	Location	Lat. and Long.	Assessments made
S21S8641055	1986	60 (18 sampled)	Bulstofta (Sweden)	56° 0´N, 13° 0´E	Wood quality, growth and phenology (**III**)
S21S8741083	1987	60 (18 sampled)	Ingelstad (Sweden)	56°43´N, 14°54´E	Wood quality, growth and phenology (**III**)
21202 / 2	1998	25	Lohja (Finland)	60°21´N, 24°07´E	Growth (**IV**)
21202 / 3	1998	25	Lohja (Finland)	61°09´N, 24°00´E	Growth (**IV**)
21202 / 4	1998	25	Hyvinkää (Finland)	60°37´N, 25°04´E	Growth (**IV**)
2071 / 1	1998	25	Loppi (Finland)	60°39´N, 24°29´E	Growth (**IV**)
1811	1994	5	Viikki (Finland)	60°14´N, 25°05´E	Morphology, physiology and phenology (**I, II**)

3.2. Methods

Studies have been made on: (1) yield related characters: photosynthesis, leaf and stomatal dimensions, phenology and growth rate, (2) wood quality related characters: alkali soluble lignins, fibre length, coarseness, fibre count and form factor, (3) characters and aspects related to mass vegetative propagation.

More specifically, the following studies were made:

--Observations on phenology (Paper I, III)

Growth initiation was taken as the number of days when 70% (± 5%) of the buds on the observed branches of a tree were judged to have emerged 5 mm beyond the bud scale. Days were counted from the first of April (III) or first of May (I). Growth cessation was the day when 50% (±10%) of the observable leaves were judged as having yellowed, counting from the first of September (I) or October (III). The length of the growth period was estimated as the number of days from growth initiation to growth cessation.

--Measurements of growth parameters (Paper I, III)

Total height, basal diameter 15 cm from the ground and breast diameter at height 1.3m were measured. For trees under age five, stem volume was estimated as height x basal diameter^2 (Ceulemans et al. 1992; Li et al. 1998). For the large trees in the Swedish trials, stem volume was estimated according to Eriksson (1973).

--Measurements of physiological parameters (Paper II)

Net photosynthesis and related CO_2 exchange parameters, including stomatal conductance, intercellular CO_2 concentration, CO_2 resistance and transpiration by the leaves were measured. For the leaf characters, we measured total nitrogen of the leaves, leaf size, shape (width/length), fresh weight/area and dry weight/area. The leaf stomatal characters stomatal density (no/mm^2) and guard cell length (μm) of fresh leaves were also observed.

--Measurements of wood quality (Paper III)

The measurement of alkali soluble lignin was described by Rautio (1999). Fibre properties related to paper making were analyzed with a Kajaani FS-200 fibre analyzer. The results were obtained in terms of coarseness (C in mg/m), arithmetic average fibre length (Aafl in mm: $\sum n_i l_i / \sum n_i$), and length-weighted average fibre length (Lwfl in mm: $\sum n_i l_i^2 / \sum n_i l_i$). Fibres are grouped into various length classes, and n_i is the number of fibres in length class l_i. On the basis of these values, a fibre count (1/C**Lwfl*, in 10^6 fibres/g pulp) and a form factor ($Lwfl^{\,2}$/C) were calculated (Levlin and Söderhjelm 1999).

--Vegetative propagation (Paper V)

For vegetative propagation experiments, the following traits associated with rooting were measured: rooting percentage, rooting time, length of the longest root, number of roots, presence of laterals, presence of callus around the base of cuttings, length of axillary shoot of stem cutting, height of the cutting plant, and number of cuttings obtained from one donor plant in 30 days.

3.3. Statistical analyses

The statistical analyses were performed with the SAS software package. Morphological and physiological traits were tested for significance with analysis of variance (ANOVA) using the Proc GLM of the SAS Package. Means were separated and ranked using Tukey's Honestly Significant Difference (**I**, **II** and **V**). Stability analyses were computed by applying a regression analysis, estimating deviation from regression and ecovalence (**IV**).

Variance components were calculated by using the appropriate mean square values and corresponding expected mean square coefficients generated from PROC GLM; RANDOM/TEST option (SAS Inc. 1990) (**III** and **IV**). The MANOVA statement of the SAS PROC GLM provided the sum of cross products for estimation of covariance (**III**). Inter-trait genetic correlations were estimated using the formulae in paper **IV**. Inter-site genetic correlations were estimated following Burdon (1977) (**III**). SAS Proc CORR was used to calculate Pearson's product-moment correlation (**I**, **II**, **III**, **IV** and **V**).

4. Results and Discussion

4.1. Yield components

Improvements in wood quality and growth are key criteria for breeding and selection. Identifying superior genotypes in early ages should render short-rotation silviculture more efficient. Poplar yield increments are affected by many components related to morphology, physiology and phenology (e.g. Cain and Ormrod 1984; Ceulemans et al. 1992; Thomas et al. 1997a, 1997b). In this thesis, morphological, physiological and phenological traits are considered as yield components.

4.1.1. Morphology

High levels of clonal variation have been found for morphological traits in trembling aspen (Reighard and Hanover, 1990; Thomas et al. 1997a, 1997b). Tenable explanations for the superior performance of the hybrid material can only be based on better analysis and insight into determinant traits related to growth.

In a study of two hybrids (*P. x euramericana*) and two clones of poplars (*P. deltoides* and *P. nigra*), no significant differences were found for the leaf ratio mass/area (Cain and Ormrod 1984). However, Orlovic et al. (1998) found that leaf area was strongly correlated with height among hybrid poplars. In our study, aspen hybrid clones that showed differences in growth could not be separated on the basis of leaf area or mass (II).

Variations in stomatal density and mean guard cell length might reflect differences in plant growth among genotypes. A positive correlation was found between stomatal density and fast growth in *Betula pendula* Roth (Wang et al. 1995) and *Azadirachta indica A.* Juss (Kundu and Tigerstedt 1998). Likewise, a strong correlation was demonstrated between the number of stomata on the leaf adaxial surface and biomass in *Populus* hybrids (Orlovic et al. 1998). However, Ceulemans et al. (1987) found no correlation between stomatal density (either adaxial or abaxial) and growth for hybrid poplar among *P. deltoides, P. trichocarpa* and *P. maximowiczii*. Leaf anatomical characters are affected by variations in the light regime (Garcia Nunez et al. 1995). At high light intensities, stomatal density normally increased with the time the leaves are exposed to continuous light (Zacchini et al. 1997; Koike et al. 1998).

In our study stomatal density was correlated negatively with height, but decreased linearly with mean guard cell length (II). The phenomenon is known for many plants, e.g. oak trees (Abrams and Kubiske 1990) and peach (Loreti, et al. 1993). Since stomatal density increased as fast as the mean guard cell length decreased, there was no significant difference between mean total guard cell length for four hybrid clones and the corresponding length for *P. tremula* (II). Nonetheless, significant differences existed between hybrids and *P. tremula* for mean guard cell length and stomatal density. The hybrid clones had a lower stomatal density but a larger mean guard cell length compared to *P. tremula*. (II). The high stomatal density and small mean guard cell length in *P. tremula* may be associated with the adaptation of this species to long day-lengths, or rather short night-lengths during the summer.

Broad-sense heritability for height has been estimated for trembling aspen as 0.52 (Van Buijtenen et al. 1959), 0.45 (Barnes 1969) and 0.69 (Einspahr et al. 1967). In triploid trembling aspen, 0.3 was reported (Einspahr et al., 1963). Broad sense heritability estimates for diameter in the literatures range from 0.14 (Van Buijtenen et al. 1959), to 0.36 (Barnes 1969) and 0.45 (Einspahr et al. 1967). In our study, repeatability estimates are reasonably consistent with those presented in the literature, despite the inherent variability of these estimates across different studies and populations of different ages. The repeatability estimates across two sites were 0.54 for diameter at breast height and 0.65 for height (III). Across four sites at age four, repeatabilities were 0. 51 for basal diameter and 0.60 for height (IV).

In the present study, significant variations were found in a wide array of growth, stomatal and leaf traits among the hybrid clones (I-IV). The existence of such variation indicates that clones could rather easily be selected for further breeding and practical cultivation. In addition, the analyses of clonal mean repeatabilities suggest that moderate genetic responses could be expected for growth, following clonal selection and subsequent vegetative propagation of selected phenotypes for industrial planting.

4.1.2. Physiology

In the framework recommended by Cannel (1989) for analyzing yield components, the rate of photosynthesis is assumed to influence light use efficiency. Therefore, genetic variation in leaf photosynthetic properties may strongly affect yield. Studies showed that physiological measures could be used to select superior genotypes (Ceulemans and Impens 1983, Ceulemans et al. 1987, Orlovic, et al. 1998).

However, the correlations between net photosynthesis and growth have proved ambiguous (Barigah et al. 1994, Gatherum et al. 1967, Okafo and Hanover 1978, Reighard and Hanover 1990). Most physiological studies have been made in greenhouses, or based on 1-2 year old plants in the field (Ceulemans et al, 1987, Orlovic et, al. 1998). The correlation between performance in the greenhouse and in the field has variously proved significant (Ceulemans et al. 1987) or non-significant (Thomas et al. 1997a).

For trembling aspen clones in growth chamber experiments, Thomas et al. (1997a) showed a positive relationship between net photosynthesis and dry mass or height growth, but correlations were not significant between physiological characters measured in the growth chamber and field measurements of either physiological or growth characters. Our results showed that net photosynthesis was not correlated with height, breast diameter or basal diameter (II).

Foliar nitrogen has proved a good predictor of net photosynthesis (Reich et al. 1995). There is known to be a biochemical basis for the relationship between foliar N and net photosynthesis (Evans 1989). The photosynthetic capacity of leaves is related to the nitrogen content primarily because the proteins of the Calvin cycle and thylakoids comprise the bulk of leaf nitrogen. For C_3 species, Reich et al. (1995) described the relationship between photosynthetic capacity and leaf nitrogen concentration with one general equation. In our study (II), a significant correlation was found between foliar nitrogen and net photosynthesis among the four hybrid clones. This indicates that foliar nitrogen might be used to predict net photosynthesis in aspen.

4.1.3. Phenology

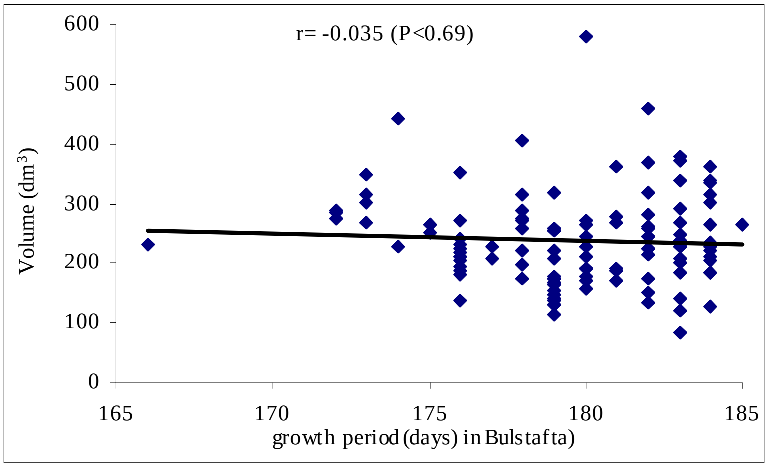
It is important to understand the effects of phenological traits on growth, because these effects are crucial for the choice of breeding strategies. High correlations between growth and phenological traits have been reported for many tree species, such as *Betula pendula* (Wang and Tigerstedt 1996), *P. trichocarpa* x *P. deltoides* (Ceulemans et al. 1992), and several larch species included their full-sib families (Baltunis and Greenwood 1999). The diversity and correlations mentioned above suggest that such phenological characters can be altered by evolutionary forces to allow adaptation to changing environments, or could be manipulated by breeders seeking yield improvement (Beuker 1994). Conversely, selection for higher yield may also inadvertently lead to a prolonged growth period, either by early growth initiation or late growth cessation, thereby incurring a higher risk of frost injury.

The results of the present study demonstrated significant differences in growth initiation, growth cessation and growth period among hybrid clones (I, III) and between hybrid clones and native *P. tremula* (I). The growth period varied from 143-158 days for the four hybrid clones, and was 112 days for *P. tremula* (I). The correlation between growth period and yield was highly significant. The annual growth rate of height for the hybrids was 4.2 cm per 7days (2.4 for *P. tremula*) in the 3rd year and 6.4 cm per 7 days (2.9 for *P. tremula*) in the 4th year. After 5 years, mean estimated stem volume of the hybrids was 3.9 times that of *P. tremula*.

The growth rate of *P. tremula* normally showed a peak in mid-June (I), and thereafter decreased constantly through the rest of the growth period. However, the hybrids normally had another growth peak in mid-August, responding strongly to a period of warm weather in the late summer. This may indicate that the hybrid genomes are adapted to different parental environments. The late flushing and early cessation of the local aspen is considered to be an adaptation to the short summers of the far north.

Ceulmans et al. (1992) noted a correlation between growth period and stem volume in hybrid poplar clones (*P. trichocarpa* x *P. deltoids*), suggesting that phenological characters can only partly explain observed differences in growth. In our study (I), there was a strong correlation between growth period and stem growth, which indicates that the fast growth of hybrids can be largely explained by their ability to utilize the extended growth period. However, when *P. tremula* was removed from the data, there was no correlation between growth period and yield of the hybrid material.

In our other study (III) at Ingelstad, there was a significant correlation between growth period and stem volume among 18 hybrid clones based on individual tree measurements, but not at the Bulstofta trial (Figure 3). The difference between the two parallel trials may be due to climatic or edaphic conditions or to stand density.



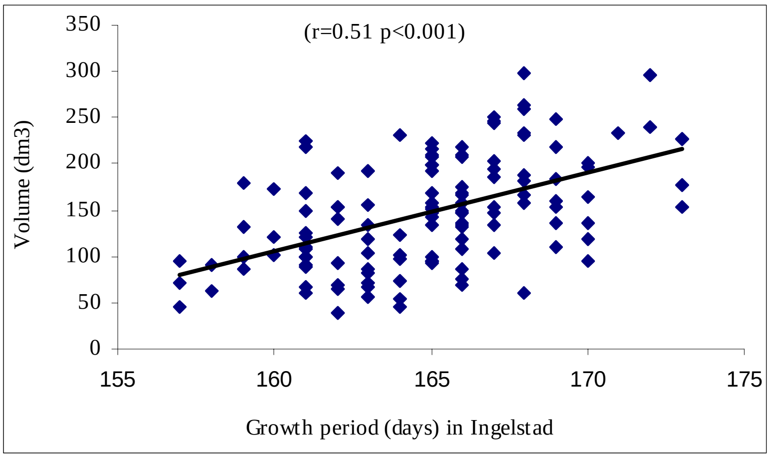


Figure 3. Correlation between growth period and stem volume in Bulstofta and Ingelstad

4.2. Wood quality components

4.2.1. Alkali soluble lignin

Broad sense heritabilities for lignin content were moderate to large, typically exceeding those for growth rate in poplars (Dinus et al. 2001). In *Eucalyptus grandis* hybrid clones, broad sense heritabilities was estimated as 0.82 on a clonal mean basis (Bertolucci et al. 1995). Our study indicates that it should be relatively easy to select aspen hybrid clones with less alkali soluble lignin, since this character has a rather high repeatability (0.85). In addition, alkali soluble lignin is genetically correlated negatively with fibre count and weakly with other fibre and growth traits (III). Improvement based on high fibre count alone could reduce lignin at the same time.

Clone x site interaction for lignin content was significant for *Eucalyptus grandis* hybrid clones (Bertolucci et al. 1995). We obtained similar results, although only two sites were used for testing (III). More and better information on the genetics of lignin content, especially as concerns relationships with growth and fibre properties and interactions across environments, would be helpful for genetic selection to improve traits for the paper industry.

4.2.2. Fibre properties

Fibre length is a wood quality character of central importance for the pulp industry (Horn 1978, Amidon 1981). There are more studies on wood density in forest tree species than on fibre properties, probably because fibre properties are more expensive to measure. For eucalyptus clones (*E. grandis*), basic wood or chip density appeared be a good indicator of fibre morphology and a measure of the potential for fibre collapse and conformability (Arbuthnot 2000). For trembling aspen, Panshin and DeZeeuw (1980) reported fibre lengths ranging from 1.32 to 1.38 mm. Yanchuk et al. (1984) reported fibre lengths from 0.67 to 0.97 mm in trembling aspen clones. They also found significant clonal differences for both wood density and fibre length.

Our arithmetic measurements of average fibre length for hybrid aspen ranged from 0.34 to 0.70 mm (III). The lower values of Aafl are probably due to fibre curling. Thus analysis with the Kajaani FS-200 instrument generally results in somewhat shorter fibre lengths than in reality. Our study showed strong clonal within family effects for growth and wood properties. The repeatability estimates across the two sites were 0.78 and 0.67 for fibre length and coarseness, respectively. There were significant differences among clones for most traits, whether the sites were considered separately or combined. However, even when significant, clonal within family differences accounted for only a small proportion of the total phenotypic variation.

The results indicate that significant improvement for wood quality and growth traits is possible in hybrid aspen clones, but care must taken in determining the size of the planting zone. The question of the number of clones used has been discussed by Bishir and Roberds (1997).

4.3.Relationship between growth and wood quality

It is important to understand the variability of forest trees for physico-chemical properties and their relationship with growth, if one is to increase the productivity and value of paper industry. Wei and Borralho (1997) found that wood density had weak unfavourable genetic correlations with diameter, height and volume growth in *Eucalyptus urophylla*. King et al. (1998) investigated the relationship between growth and fibre properties and the potential for using genetic selection to improve traits that will increase both the yield and the value of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.).

The phenotypic correlation will be generally lower than corresponding genetic correlation since clonal means includes contributions from measurement error and environmental variance (Lynch and Walsh 1998). In our study, genetic correlation coefficients between wood and growth traits followed the corresponding phenotypic correlation coefficients but were generally stronger (III). Similar results were found in a study of relationships between wood density, growth and growth rhythm traits in Norway spruce (Skrøppa et al. 1999). The presence of genotype x environment interactions will lower the phenotypic correlation. A low correlation, however, should not be interpreted as the presence of an interaction (Lynch and Walsh 1998).

Annual growth rhythm is an important determinant of variations in wood physico-chemical properties (III). An extension of the growth period would increase the length of fibres and decrease their number. The strong relationships obtained between fibre count and phenological traits of clones suggest that the latter traits could be good predictors of clonal differences in fibre count.

In our study, wood quality traits were more similar across sites than growth traits. The inter-site genetic correlations for wood characters were stronger than for growth characters (III). This was expected, as Zobel and Jett (1995) reported that genotype x environment interaction for wood properties is small, except where grossly differing environments are involved. My results suggest that selection for industrially valuable characters in Bulstofta and Ingelstad could be carried out in one carefully planned trial (III).

In our study, fibre count is currently considered as a target trait with potential importance for pulp production. In general, an increase in tree growth resulted in a slight increase in fibre length, wood coarseness and decreasing fibre count (III). Genetic improvement of hybrid growth should therefore, probably result in an increase of coarseness and lower fibre count.

4.4. Genotype x environment interaction

Geneotype x environment (G x E) interaction is generally defined as the differential performance of genotypes among environments (Falconer 1970). More important to forest tree breeding is the predictability of yield of a genotype in various environments. This concept of stability could be measured by the regression coefficient, ecovalence and deviation mean squares from the regression line.

The regression coefficient measures relative performance. Genetic correlations of the same traits expressed in different environments can be used to identify the extent to which a given genotype penetrates similarly in different environments (Falconer 1970). In forest tree breeding this information is useful to distinguish genotypes for specific environments. This was the criterion we used to carry out selection for character stability (IV).

In our study, there was a high correlation for height increment during the third year between two trials planted on agricultural land, although the site mean was very different for the two sites. However, there was no correlation between the two forest land trials. The reason for the correlation between the two sites on agricultural land probably lies in the uniform soil types of such terrain.

The genetic gain was 17% for height and 15% for basal diameter above the overall mean (IV). These gain estimates can be achieved from further clonal selection, and do not include gains already achieved from past selection. In eastern cottonwood (*Populus deltoides*), genetic gain was 9 % for height and 21% for diameter when the top four clones were selected out of 32 clones (Randall and Cooper 1973).

In our study (IV), expected gains at planting sites were generally greatest or nearly so, when selection was done at the same site. When selection is done at a single site for planting at all sites, the best site for testing is one with highest site mean, the largest within-site repeatability and the greatest variances between clones.

4.5. Vegetative propagation (V)

Propagation costs could be cut by replacing part of the micropropagation process with steps involving more traditional techniques. This study explored ways of improving existing vegetative propagation techniques for aspen by using stem cuttings obtained from micropropagated plants.

Noh (1988) investigated the potential of rooting ability of nodal cuttings derived from micropropagated aspen (*P. davidiana* Dode) in the greenhouse. He reported a rooting percentage of 85% in the best substrates (peat 1: vermiculite 1: perlite 1). In our study, cuttings containing one axillary bud were harvested from the same donor plants twice during the growing season in May and July. The average rooting percentage was 53% in the first harvest and 27% in the second. A significant variation for ten traits related to rooting ability was found among the clones, indicating that clonal effects play an important role in the propagation by stem cutting of aspen.

Shoots that sprout from the mature stump close to the root system are usually considered to be the most responsive plant material in vegetative propagation (Bonga 1982). In our study, two of the clones were of the same age, 32 years. One clone A, which initially originated from root suckers, rooted no better than its full-sib clone B initiated from branch buds. Root suckers are considered to represent juvenile tissue. We tentatively suggest that in the case of easily rooted clones, juvenility probably has little effect on the rooting ability. Both clones rooted well in both harvests.

In most cases, an exogenous application of auxin is needed to induce good root formation in cuttings (Loach 1988). Clones A and B rooted more easily than L or M, even without IBA treatments. Rooting percentage was only slightly, and not

significantly, improved by 0.6 mM IBA and 1.2 mM IBA in the first harvest, but was significantly improved by 1.2 mM IBA in the second harvest. Our tentative hypothesis is that aspen hybrids may have a favorable concentration of endogenous auxin during early summer, but later in the summer some hybrids with an especially low level of cell auxin would respond to auxin treatment.
The ranking of clones for rooting percentage was not consistent between the first and second harvest. Houle and Babeux (1993) obtained higher rooting success with cuttings of *Populus balsamifera* and *Salix planifolia* when the trees were sampled in May and June than in July. Lanphear and Meahl (1963) reported that conditions stimulating active growth inhibit rooting and that inhibition of growth is often associated with increased rooting ability of cuttings. The other reason for the difference in rooting percentage between the two harvests may lie in large differences in the rooting environment. The environment in the greenhouse between the first harvest and the second harvest had changed considerably, since the daylength and thermoperiod changed as the growing season progressed.

The results obtained in the present study tend to suggest that stem cuttings could be harvested twice during the same growing season, but economic utilisation of the second harvest remains uncertain. Compared to the average rooting percentage (90%) from micropropagation in the laboratory at Haapastensyrjä, overall rooting percentage of aspen propagated by stem cuttings in both harvests was rather low (40%). In order to improve survival of the cutting plants and increase production of cuttings, further research is needed to improve the root system and the number of cuttings per donor plant.

4.6. Breeding implications

4.6.1. Yield components as selection criteria

Variations in stomatal density and mean guard cell length might positively correlate with plant growth (Wang et al. 1995; Kundu and Tigerstedt 1998). However, in the present study (II), we found that height correlated negatively with stomatal density among four hybrid clones. Among the physiological and morphological characters examined here, only stomatal characters of hybrid clones differ from these of *P. tremula*. Most leaf traits failed to correlate with growth traits. In our study, there were no significant differences among the clones for leaf area, leaf fresh or leaf dry mass.

Yield is a complex character, and may well be determined by many factors including the growth period. Li et al (1998) noted that hybrid vigour in aspen hybrids (*P. tremula x P.tremuloides*) was associated with delayed bud set resulting in longer duration of growth in the first year. However, by the second year, no relationship was found between bud set and stem growth in either the intra- or inter-specific crosses. Late active growth may render the hybrids liable to frost damage in autumn and winter (Li and Adams 1993; Li et al. 1998). Li et al. (1998) propose the use of local aspen as female parents in intraspecific crosses, in order to improve frost hardiness of the hybrids. Our studies suggested that the fast overall growth of aspen hybrids is largely explained by their longer vegetative period and a strong growth response to warm weather in the late summer (I). This response is not true heterosis as has been commonly assumed in hybrid aspen (Li and Wu 1996, 1997; Li et al. 1998).

Breeding for fast growth using aspen hybrid clones is then mainly an effort to tailor the growth rhythm of the hybrid clones to match the climate in a particular zone or region. In the present case, we wish to tailor the growth rhythm of clones to the semi-maritime climates of Sweden or Finland. The natural adaptation of aspen to the region does not fully utilize the length of vegetative period. Aspen hybrid clones may be seen as typical plant cultivars whose high yields have eventually to be assessed on a risk basis. It seems likely, however, that we may secure a yield doubling over native aspen without seriously incurring risks due to cold damage in cultivation (I).

4.6.2. Wood quality components as selection criteria

The wide variation for fibre length among clones within family observed in this study suggests that there are good opportunities for influencing fibre length through selection of clonal material (III). The presented results indicated that the annual growth rhythm is an important determinant of the variation in wood physico-chemical traits of clones. An extension of the growth period would increase the length of fibres at the expense of a lower fibre count. Phenological traits of clones could be good predictors of clonal differences in wood quality traits e.g. fibre count.

Table 2. Expected response ($\Delta G/\bar{x}$) in wood quality when different selection criteria of growth and phenological traits are used.

Selection criterion	Response (%)					
	Alkali soluble lignin (%)	Aafl (mm)	Lwfl (mm)	Coarseness (mg/m)	Roughness	Fiber count (10^6 fibres/g pulp)
DBH (cm)	-2.9	1.8	1.8	1.5	1.7	-1.0
Height (m)	5.2	1.7	2.2	3.7	1.4	-4.8
Volume (dm^3)	-1.3	2.4	2.0	2.0	1.7	-2.2
Growth initiation (days)	-2.2	-0.4	-2.2	-0.6	-3.2	3.1
Growth cessation (days)	7.4	4.1	4.3	4.3	3.7	-6.8
Growth period (days)	6.9	3.1	4.1	3.2	6.2	-6.7

Response based on clonal selection at selection intensity I =1.585 (two clones out of 18); Aafl = arithmetic average fiber length; Lwfl = length-weighted average fibre length.

The expected responses from various combinations of wood quality and growth/phenology are presented in Table 2. Expected responses are generally greatest for wood quality properties when selection is based on phenological traits. Selections made for growth traits result in increasing gains for fibre length (Aalf or Lwfl) and coarseness, but decreasing fibre count.

DeBell et al. (1998) reported that fibre length differed significantly among hybrid clones between *P. trichocarpa* and *P. deltoides* and increased with ring age. Their results indicate opportunities of influencing fibre length through selection of clonal material and by choice of rotation age. In our study (III), fibre length differed significantly among clones within family, and fibre length increased with growth. This is an unfavorable correlation from a breeding perspective. However, the magnitudes of the estimates were moderate, indicating that it may be possible to find genotypes with favorable combinations of desirable traits, so called correlation breakers. Further studies need to establish the relationship between silvicultural treatments (e.g. planting space, rotation age and nutrition supply) and fibre length/coarseness in hybrid aspen clones.

4.6.3. Testing and selecting

A statistically significant interaction in height growth exists between aspen clones and their test sites in southern Finland (IV). Since the four trials were all located in the same region, temperature and rainfall are not likely to be the main factors for determining the clone x site interaction. The slope of the terrain, soil structure and nutrient availability probably constitute environmental effects that may interact with genotypes. This interaction may be reduced by dividing the region into planting zones on the basis of type of terrain and soil type. This is expected to result in an increase in gain from clonal selection, within each soil type and site condition. Interactions may also be reduced by selecting stable clones. The gain from a reduction in clone x site interaction must be compared with the loss that results when clones of high growth potential on specific sites are excluded. Whether one or two planting zones are used, the best sites for testing within each zone may be identified using the repeatability at the respective sites.

The next step would be to identify the environmental characteristics that determine a good testing site for growth and wood quality. Identification of specific environmental conditions involved in generating interactions can lead to selection of clones suited to specific environments. Such a procedure applied within a clonal programme would involve a massing of a considerable body of records, and the resulting increase in costs would need to be weighed against possible gains.

Choosing the traits defining the best genotypes for use as parents in advanced generation breeding is a critical step in any forest tree breeding programme. Many attributes of a tree can be measured, but for selection to be most effective, the number of traits must be limited to those few that significantly influence the yield and quality of desired wood products. This requires a knowledge of end-use product requirements and of how tree characters and wood properties affect those requirements.

The high cost of trait measurements has long impeded efforts to improve wood chemical and physical properties. Wood volume per tree can be measured in minutes for pennies per tree. In contrast, traditional wet chemistry assays of lignin content take several days and cost $500 or more per sample (Dinus et al. 2001). Cost is a particularly serious problem in classic selection and breeding because the approach involves testing large numbers of clones or families for screening.

The utility of physiological selection criteria in poplar breeding is contingent on the hypothesis that genetic selection for physiological yield components is more effective than univariate selection for an integrated trait such as stem height, diameter or volume. Riemenschineider et al. (1992) think that the hypothesis remains essentially untested. Many physiological criteria simply cannot be measured on all trees in large breeding populations. Thus, it is generally unclear how physiological selection criteria might be utilized in poplar breeding. Although there are some correlations among physiological traits (e.g. photosynthesis and leaf nitrogen), they are not relevant to the need of growth in the field. According to my knowledge, physiological traits have scarcely ever been used in real practical applications of selection.

The most effective means of selecting to improve overall merit is to combine information on the economic importance of traits and their genetic potential (Hazel and Lush 1943). In order to selected superior clones, an index assigning appropriate economic weights to fibre count, form factor, coarseness, alkali soluble lignin and growth should be defined, with the exact coefficients dependent on the specific breeding objectives for pulp production.

In addition to growth, the selection index must consider at the most 2-3 industrially important traits, each weighted on the basis of its genetic and economic significance. However, it is uncertain how economic weights should be determined (Cotterill and Jackson 1985). There are only a few examples where relative economic weights based on product value have been reported for commercial tree species, because product recovery studies are expensive and labor intensive (e.g. Cotterill and Jackson 1985, Aubry et al. 1998). No product value based economical weights have been reported previously for hybrid aspen clones. I believe that such economic weights of these traits could be formulated on the basis of the present study, in collaboration with paper industry specialists.

An important question in clonal forestry concerns the number of clones needed in plantations to protect against catastrophic failure, while at the same time achieving uniform stands, high yields, and ease of management. Examples of catastrophes in Europe include the outbreak of *Venturia populina* in poplar plantations in Italy during the 1930's, and the devastation caused in the 1970's by the spread of *Marssonina brunea* through poplar stands following widespread monoclonal planting of clone I-214 (Heybroek 1978; Zsuffa et al. 1993). Attempts to control the risk associated with clonal plantations were legislative, e.g. Sweden and Germany mandated minimum numbers of clones to be used, the numbers ranging from 20 to over 100 depending on species and other considerations (Hedström and Krutzsch 1982; Muhs 1993).

In Finland, a total of 27 aspen hybrid clones have been planted over a total area of 270 ha in southern Finland since 1997. It has been planned to increase aspen cultivation by 500 ha per year in the near future. The use of very large number of clones appears not only unattractive commercially, but unnecessary. Bishir and Roberds (1997) opined level of risk is unlikely to change significantly after the number of clones used exceeds about 30 or 40.

4.6.4. Suggested breeding strategies

The Finnish hybrid aspen breeding programme was started 50 years ago, because the match industry was interested in aspen and poplar wood. The breeding goal at that time was for high yield and good stem “outer quality” as determined by the match industry. No attention was paid to wood chemical properties. In the meantime, the old field trials have degenerated and origins of most of the original hybrid parents are obscure. A similar programme was started in Sweden about ten years earlier, and most of the wood quality analyses in this study (III) come from Swedish clone trials. A joint breeding strategy for both countries could now be most effective.

The future aspen breeding programme would focus on the interspecific hybrids of European aspen with American trembling aspen. A simple recurrent selection system for specific combining ability (SCA) would be used. To begin with, plus trees of *P .tremula* and *P. tremuloides* could be selected from Finland, Sweden and North America on the basis of yield, stem and wood quality. These plus trees would consist of about 25 females and 25 males, and would comprise a total of about 50 individuals, representing both species in equal amounts.

A single pair and a factorial mating design could be followed to identify within and between superior species combinations. In the meantime, a factorial mating design would be used to estimate of genetic parameters, e.g. general (GCA) and specific combining ability (SCA) of the parents. Single pair matings with genetically proven parents would produce breeding populations for advanced generation selection (Zobel and Talbert 1984). The total time for one rotation of selection would be less then 20 years.

Field tests of hybrid progenies would include both unimproved check lots and the best material selected from old hybrid trials in Finland and Sweden. This would provide information on hybrid superiority (comparisons with pure aspen), genetic gain (comparison with checklots) and the best source of combination for hybridization (Li 1995). Additional field tests could be set up, and hybrid progeny performance analyzed to identify complementary aspen parents that provide maximum hybrid vigour. On the basis of the performance of the hybrids, a positive assortative mating may be used with the selected parents to create new materials for hybridization in future generations.

A flow chart for breeding *P. tremula* and *tremuloides* hybrids in Finland is suggested in Figure 4.

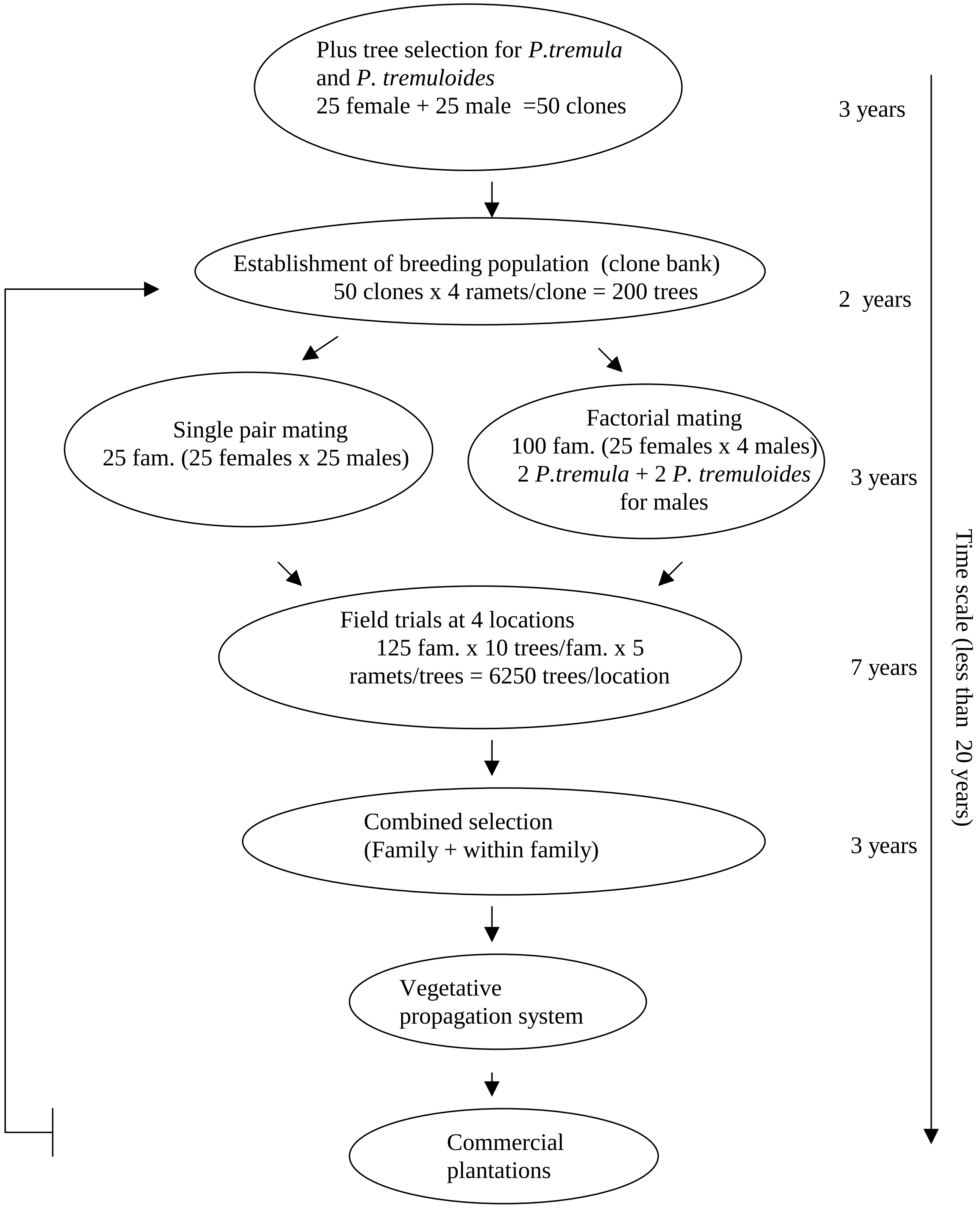


Figure 4. A flow chart for hybrid aspen in industrial plantation forestry at one planting zone.

5. Conclusions

The hybrid aspen clones showed significantly higher growth rates than *P. tremula* over the entire growth season. The growth rate of *P. tremula* normally had one peak in mid-June. It then decreased constantly through the rest of the growth period. However, the hybrids normally had another growth peak in mid-August, responding strongly to a period of warm weather in the late summer. This may indicate that the hybrid genomes are adapted to two different parental environments. The late flushing and early cessation of the local aspen is considered to be an adaptation to the short summers in the far north. The fast overall growth of the aspen hybrids is largely explained by their longer vegetative period.

Large variations in physiological, morphological, phenological traits and wood physico-chemical properties, as well as in rooting ability, were found among hybrid clones. Yield components might be controlled by many genes, specific to each clone. No single gas exchange or morphological variable can provide a reliable indicator of yield potential.

It appears to be relatively easy to select individuals superior for improving wood physico-chemical properties and growth since these properties have rather high repeatabilities. However, improvement based on fibre count alone could reduce growth. Given the high repeatabilities of the alkali soluble lignin content, opportunities for reducing this character in hybrid aspen clones are good, especially when alkali soluble lignin is only weakly correlated with other traits, or negatively correlated with fibre count genetically.

Fibre length increased with growth. This is an unfavorable correlation from our breeding perspective in this particular case, because special high quality papers need uniform short fibres. Further work would be needed to establish the relationship between silvicultural treatments (e.g. planting space, rotation age and nutrition supply) and fibre length/coarseness in hybrid aspen clones.

A statistically significant interaction in height growth exists between hybrid aspen clones and their test sites. This interaction may be reduced by selecting stable clones. Stratification of areas into planting zones would reduce the interaction based on site topography and soils. The gain from a reduction in clone x site interaction should be compared with the loss from excluding clones of high growth potential. Whatever method is used, the best sites for testing within each zone may be identified using the clonal mean correlations, taking into account the repeatability at the respective sites.

High genetic gains can be achieved by cloning aspen hybrids with desirable yield and quality. However, cloning costs are high, no matter what propagation system is used. Industrially superior clones can be selected on the basis of both yield and quality parameters, which can be combined to provide a meaningful selection index. We now need to focus our efforts in order to devise a propagation system that can transfer genetic gains to economically sound cultivation systems.

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